Mycobacterium cosmeticum sp. nov., a novel rapidly growing species isolated from a cosmetic infection and from a nail salon

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Four isolates of a rapidly growing *Mycobacterium* species had a mycolic acid pattern similar to that of *Mycobacterium smegmatis*, as determined by HPLC analyses. Three of the isolates were from footbath drains and a sink at a nail salon located in Atlanta, GA, USA; the fourth was obtained from a granulomatous subdermal lesion of a female patient in Venezuela who was undergoing mesotherapy. By random amplified polymorphic DNA electrophoresis and PFGE of large restriction fragments, the three isolates from the nail salon were shown to be the same strain but different from the strain from the patient in Venezuela. Polymorphisms in regions of the *rpoB*, *hsp65* and 16S rRNA genes that were shown to be useful for species identification matched for the two strains but were different from those of other *Mycobacterium* species. The 16S rRNA gene sequence placed the strains in a taxonomic group along with *Mycobacterium frederiksbergense*, *Mycobacterium hodleri*, *Mycobacterium diernhoferi* and *Mycobacterium neoaurum*. The strains produced a pale-yellow pigment when grown in the dark at the optimal temperature of 35 °C. Biochemical testing showed that the strains were positive for iron uptake, nitrate reduction and utilization of D-mannitol, D-xylose, iso-myo-inositol, L-arabinose, citrate and D-trehalose. The strains were negative for D-sorbitol utilization and production of niacin and 3-day arylsulfatase, although arylsulfatase activity was observed after 14 days. The isolates grew on MacConkey agar without crystal violet but not on media containing 5 % (w/v) NaCl or at 45 °C. They were susceptible to ciprofloxacin, amikacin, tobramycin, cefoxitin, clarithromycin, doxycycline, sulfamethoxazole and imipenem. The name *Mycobacterium cosmeticum* sp. nov. is proposed for this novel species; two strains, LTA-388^T (= ATCC BAA-878^T = CIP 108170^T) (the type strain) and 2003-11-06 (= ATCC BAA-879 = CIP 108169) have been designated, respectively, for the strains of the patient in Venezuela and from the nail salon in Atlanta, GA, USA.

Non-tuberculous mycobacteria (NTM) were first reported in pathological human secretions in 1884 (Bloom, 1885),

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Abbreviations: NTM, non-tuberculous mycobacteria; PRA, PCR restriction analysis; RAPD, random amplified polymorphic DNA; RGM, rapidly growing *Mycobacterium*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *hsp65* gene sequences of strain LTA-388^T and the 16S rRNA and *hsp65* gene sequences of strain 2003-11-06 are AY449728–AY449731, respectively.

A micrograph showing acid-fast bacilli, a photograph showing colonies of strain LTA-388^T and photographs showing *hsp65* PRA patterns and PFGE and RAPD typing patterns are available as supplementary material in IJSEM Online.

only 2 years after Koch reported the tubercle bacillus. Prior to the early 1980s, reports of diseases caused by NTM were somewhat sporadic and the diseases were often associated with environmental sources (Falkingham, 1996). Skin and wound infections following invasive procedures are relatively rare ($\leq 5\%$ of procedures) and NTM are involved only occasionally (Murillo *et al.*, 2000). The most common NTM species associated with primary cosmetic infections are rapidly growing *Mycobacterium* (RGM) species including *Mycobacterium chelonae*, *Mycobacterium abscessus* and *Mycobacterium fortuitum*. The NTM also appear to be gaining importance as opportunistic pathogens, particularly among immunocompromised patients in developed nations (Falkingham, 1996).

In a recent study involving a nail salon in Atlanta, GA, USA,

two cases of furunculosis caused by *Mycobacterium mageritense* were identified. Three additional NTM isolates from cultures obtained from footbaths and a sink drain were identified as '*Mycobacterium goodii*/*Mycobacterium smegmatis*' on the basis of mycolic acid patterns, determined by HPLC, showing features of both species (Gira *et al.*, 2004). A similar pattern was also observed for an isolate from a culture of a granulomatous subdermal lesion of a female patient in Venezuela who was undergoing mesotherapy with an unknown substance(s) for a cosmetic purpose (weight loss). We evaluated these isolates by using a polyphasic approach that included microscopic and macroscopic morphological examination, biochemical and antimicrobial drug-susceptibility patterns, nucleotide sequence analyses of three taxonomically useful regions of the *rpoB*, *hsp65* and 16S rRNA genes and strain typing by random amplified polymorphic DNA (RAPD) electrophoresis and PFGE of large restriction fragments. Our data indicate that the four isolates previously identified as '*M. goodii*/*M. smegmatis*' were, in fact, representative of two strains of a novel RGM species.

The isolate taken from a patient was obtained by treating material from the granulomatous lesion with *N*-acetyl-L-cysteine (0.1875%, w/v) and NaOH (1%, w/v) and inoculating Löwenstein-Jensen (LJ) medium as previously described (Kent & Kubica, 1985). The environmental isolates were obtained from swabs of sink and footbath drains, which were placed in 2 ml Middlebrook & Cohn 7H9 broth (Remel) and decontaminated with 0.005% (w/v) cetylpyridinium chloride. An aliquot (1 ml) of each broth specimen was filtered through 0.45-µm filters that were washed twice with 100 ml 0.00425% (w/v) monopotassium phosphate buffer, and incubated on Middlebrook 7H10 agar (Remel) plates containing oleic acid albumin dextrose complex enrichment at 35 °C. Mycolic acids derivatized as *p*-bromophenacyl esters were examined by using a standard UV-HPLC method (Butler *et al.*, 1996) and a model System Gold HPLC system (Beckman Coulter) equipped with a Beckman C18 reverse-phase ultrasphere-XL cartridge and a Beckman model 166 UV detector. Control strains for HPLC included *M. smegmatis* ATCC 19420^T, *M. mageritense* ATCC 700351^T, *Mycobacterium wolinskyi* ATCC 700010^T and *M. goodii* ATCC 700504^T.

Antimicrobial drug-susceptibility testing was performed in cation-supplemented Mueller-Hinton broth by using a microdilution method, and interpretations of MICs were made according to NCCLS standards for organisms that grow aerobically (NCCLS, 2002). Microscopic analysis of acid-fast bacilli following Kenyon staining, and testing for colonial morphology, pigment production, single-source carbon utilization, growth rate, tolerance of elevated incubation temperature (45 °C) or 5% (w/v) NaCl, iron uptake, niacin production, nitrate reduction, arylsulfatase production and ability to grow on MacConkey agar without crystal violet were performed as previously described (Kent & Kubica, 1985). Media and reagents for biochemical

tests were prepared at the Centers for Disease Control and Prevention (CDC), with the exception of niacin test strips and nitrate-reduction broth and reagents, which were obtained from Remel. The results of biochemical tests were read after 7 and 14 days incubation at 28 °C, except for arylsulfatase, which was read after 3 and 14 days incubation, and ability to grow on MacConkey agar without crystal violet, which was read after 5 and 11 days, as described in a standard protocol for biochemical testing of mycobacteria (Kent & Kubica, 1985). Control strains for biochemical testing included *M. smegmatis* ATCC 35797, *M. fortuitum* ATCC 6841^T, *M. chelonae* ATCC 35752^T, *Mycobacterium avium* ATCC 35713 and a BCG strain of *Mycobacterium bovis* (ATCC 35731), all of which were from the stock collection of the Mycobacteriology Branch of the CDC.

Genotypic tests were performed using DNA extracted from bacteria grown in complete Middlebrook & Cohn 7H9 broth (Remel) with a modified bead-agitation method as previously described (Cooksey *et al.*, 2003). Oligonucleotide primers for PCR restriction analysis (PRA) of the *hsp65* gene and for sequencing of regions of the *rpoB*, *hsp65* and 16S rRNA genes are shown in Table 1. Amplification of these regions by PCR was performed in 200 µl, thin-walled, flat-top PCR tubes using 25 µl HotStar Taq polymerase master mix (Qiagen), 100 nM primers, 1 µl DMSO and 1 µl template in a final volume of 50 µl. The PRA was performed using a 441 bp fragment of the *hsp65* gene, which was amplified using primers TB11 and TB12 (Telenti *et al.*, 1993). Conditions for PCR thermocycling, restriction with *Bst*EII and *Hae*III and electrophoresis were as previously described (Hernandez *et al.*, 1999; Cooksey *et al.*, 2003). Control strains for *hsp65* PRA included *Mycobacterium hodleri* DSM 44183^T, *Mycobacterium frederiksbergense* NRRL B-24126, *Mycobacterium neoaurum* ATCC 25796, *Mycobacterium diernhoferi* ATCC 19340^T, *M. smegmatis* ATCC 19420^T, *M. mageritense* ATCC 700351^T, *M. wolinskyi* ATCC 700010^T and *M. goodii* ATCC 700504^T. Sequence analysis of the 441 bp region of the *hsp65* gene was performed using a 600 bp template obtained under the same PCR conditions as for PRA, except that primers HSPF1 and HSPR1 (Table 1) were used for amplification and for sequence analyses of sense and antisense DNA strands. A 360 bp region of the *rpoB* gene that was shown to be polymorphic among 26 *Mycobacterium* species was amplified under conditions that were similar to those used for the *hsp65* gene amplification, except that the annealing temperature was 58 °C and primers RPO5' and RPO3' (Lee *et al.*, 2003) were used for amplification and sequencing reactions. A region of the 16S rRNA gene from nucleotide 8 to 1542 (*Escherichia coli* numbering) was amplified using primers pA and pH, as described previously (Edwards *et al.*, 1989; Floyd *et al.*, 1996), and sequenced by using three forward and three reverse primers (Table 1).

Amplicons that were used as templates for sequence analyses were purified by using QiaQuick columns (Qiagen) and diluted 10-fold in type 1 water. Sequencing reactions

Table 1. Oligonucleotide primers used in genotypic characterization of strains

Gene	Accession no.	Primers	Reference(s) or sequence	Nucleotides	Product (bp)	Assay
<i>rpoB</i>	P47766	RPO5'/RPO3'*	Lee <i>et al.</i> (2003)	902–1261	360	Sequence
<i>hsp65</i>	M15467	TB11/TB12*	Shinnick (1987); Telenti <i>et al.</i> (1993)	396–836	441	PRA
<i>hsp65</i>	AF456473	HSPF1/HSPR1*	tgacgcggtcaaggtgacgc/gcaggatgtagggatcctcc	81–680	600	Sequence
16S rRNA	X15916	pA/pH*	Edwards <i>et al.</i> (1989)	8–1542	1535	Sequence
16S rRNA	J01859	AIF	ctggctcaggacgaacgctg	20–39	None	Sequence
16S rRNA	J01859	8F	gtaggtggtttgtcgcgttg	581–600	None	Sequence
16S rRNA	J01859	2F	ttgggttaagtcccgaacgag	1084–1105	None	Sequence
16S rRNA	J01859	54R†	tctagtctgccgtatcgccc	654–634	None	Sequence
16S rRNA	J01859	16R†	ataaggggcatgatgacttgac	1213–1192	None	Sequence
16S rRNA	J01859	H1R†	ccagccgcaccttcggtac	1530–1511	None	Sequence

*Forward/reverse complement sequence.

†Reverse complement sequence.

contained 2.5 µl diluted amplicon, 8 µl master mix from the Dye Terminator Cycle Sequencing with Quick Start kit (Beckman Coulter), 2.0 µl 1.6 µM primer and 7.5 µl type 1 water. Cycle sequencing was performed by using a GeneAmp model 9700 thermocycler (PE Applied Biosystems) according to directions in the Dye Terminator Cycle Sequencing with Quick Start kit. Sequencing products were purified and dried using a Pellet Paint NF co-precipitant kit (Novagen), according to the manufacturer's instructions, resuspended in 40 µl sample loading buffer and then sequenced using a model CEQ 8000 fluorescence capillary sequencer (Beckman Coulter). The SEQUENCHER version 4.0 software (Gene Codes) was used for assembly of the 16S rRNA gene sequence. The sequence was compared with sequences deposited in public databases including GenBank (National Center for Biotechnology Information at <http://www.ncbi.nlm.nih.gov/>), by using the standard BLAST program (BLASTN; NCBI/NLM/NIH), and with the peer-reviewed database at Ribosomal Differentiation of Medical Micro-organisms (RIDOM) at <http://www.ridom-rdna.de/> (Harmsen *et al.*, 1999). The species with the most similarity to the 16S rRNA gene query sequences were selected for phylogenetic analysis. Similarity comparison, multiple sequence alignments and phylogenetic analyses of 16S rRNA gene sequences with pre-compiled aligned RNA sequence data at the RDP-II at Michigan State University (East Lansing, MI, USA) at <http://rdp.cme.msu.edu/index.jsp> (Cole *et al.*, 2003), were performed by using online phylogenetic programs in PHYLIP version 3.5c (Felsenstein, 1989) at the PHYLIP interface at this web site. RGM species with similar 16S rRNA gene sequences were downloaded from RDP-II for analysis, and a 16S rRNA gene sequence from *Mycobacterium tuberculosis* was selected as the evolutionary outgroup. A distance matrix was determined with the DNADIST program (Felsenstein, 1989); a phylogenetic relationship inferred with the parameter defined by Jukes & Cantor (1969), an unambiguous alignment mask, was automatically applied and a tree was

constructed using the neighbour-joining method (Saitou & Nei, 1987).

The DNA preparations used for PCRs were also utilized for typing the isolates, using RAPD electrophoresis as described previously (Cooksey *et al.*, 2003). The oligonucleotide primer used for RAPD typing was IS986-FP (5'-ACGCTCAACGCCAGAGACCA) (Linton *et al.*, 1994). Typing of isolates by using PFGE of large restriction fragments (>48 kbp) was performed as previously described (Hector *et al.*, 1992), except that DNA samples were restricted with 40 U *AseI* (Invitrogen). Samples underwent electrophoresis in 1% (w/v) agarose that was prepared and run in 0.5 × TBE buffer (0.445 M Tris/borate, 0.01 M EDTA, pH 8.3) using a contour-clamped homogeneous electric field electrophoresis/direct repeat (CHEF-DR) pulse electrophoresis system (Bio-Rad) set for 5–20 s ramping at 200 V. Thiourea was added to the running buffer (final concentration, 50 mM) to prevent DNA degradation during electrophoresis (Romling & Tummler, 2000).

Microscopic examination of acid-fast bacilli smears revealed that isolates LTA-388^T and 2003-11-06 were acid-fast, with no chains and few clumps of bacteria. The dimensions of a typical bacillus were approximately 0.55 × 1.5 µm by comparison with a microscopic micrometer (Carl Zeiss). Colonies that appeared on Middlebrook 7H10 agar plates or LJ agar slants after aerobic incubation for 3 days at either 28 or 37 °C were smooth, dome-shaped, opaque and possessed a pale-yellow pigment. A micrograph showing acid-fast bacilli and a photograph showing colonies of strain LTA-388^T are available as Supplementary Figs A and B in IJSEM Online. The isolates grew on MacConkey agar without crystal violet but not on LJ agar containing 5% (w/v) NaCl or in Middlebrook 7H9 broth at 45 °C.

Biochemical testing showed that the four isolates were positive for iron uptake, nitrate reduction, 14-day aryl-sulfatase production and for utilization of D-mannitol,

D-xylose, iso-*myo*-inositol, L-arabinose, D-trehalose and citrate. They were negative for D-sorbitol utilization and for production of niacin and 3-day arylsulfatase.

Mycolic acid patterns determined by using HPLC were identical for the three isolates from the nail salon in the USA, and this pattern was closely similar to that of the isolate of the female patient in Venezuela. The closely similar patterns of the two strains were substantially different from the patterns of other RGM species, but they most closely resembled reference mycolic acid patterns of *M. smegmatis*, *M. goodii*, *M. wolinskyi* and *M. mageritense*. The type strains of these four species were analysed concurrently with strains LTA-388^T and 2003-11-06 (Fig. 1). The most notable similarities in the mycolic acid patterns resided with the study strains. Visual comparison of mycolic acid patterns for the other species revealed major differences in the number of peaks and in peak heights, criteria that are used to distinguish mycobacterial species (Butler & Guthertz, 2001).

The isolates also had a unique *hsp65* PRA pattern in comparison with those of species included in the *M. smegmatis* group and other species included in a multicentre web site for *hsp65* PRA patterns at <http://app.chuv.ch/prasite/index.html>. A photograph showing the patterns obtained after digestion of the 441 bp *hsp65*-PCR product with *Bst*EII or *Hae*III for the four isolates along with those for *M. goodii*, *M. wolinskyi* and *M. smegmatis* is available as Supplementary Fig. C in IJSEM Online. Fragment sizes were determined subsequently by DNA sequence analysis for the four isolates: these were found to be 310 and 130 bp for *Bst*EII and 140, 96, 80, 40, 36, 32 and 16 bp for *Hae*III.

Strain-typing patterns produced by RAPD electrophoresis after amplification using primer IS986-FP and by PFGE after digestion of DNA with *Ase*I matched for isolates 2003-11-06, -24 and -30. The RAPD pattern for these

isolates consisted of two prominent bands representing approximately 450 and 320 bp. The RAPD pattern for isolate LTA-388^T consisted of multiple bands; the most prominent bands represented approximately 500 and 600 bp. The PFGE pattern for this isolate consisted of six prominent bands between 97 and 242 kbp; only two of these bands were approximately equal in size to any of the eight prominent bands in this size range for the environmental isolates. Therefore, the three isolates obtained from environmental sources at the nail salon were considered to represent one strain, and isolate LTA-388^T represented another strain. Photographs of PFGE and RAPD typing patterns are available as Supplementary Fig. D in IJSEM Online.

According to NCCLS interpretive standards, each of the four isolates was susceptible to the eight antimicrobial drugs that were tested. The MICs for the drugs were the same for each of the three isolates from the nail salon: ciprofloxacin, 0.25 µg ml⁻¹; amikacin, 4 µg ml⁻¹; imipenem, ≤1 µg ml⁻¹; tobramycin, 4 µg ml⁻¹; clarithromycin, 0.25 µg ml⁻¹; sulfamethoxazole, 8 µg ml⁻¹; doxycycline, ≤0.25 µg ml⁻¹; and cefoxitin, 8 µg ml⁻¹. These MICs were also observed for the isolate from the patient in Venezuela, except that the MIC for sulfamethoxazole was 32 µg ml⁻¹.

Sequences of portions of the *rpoB* (321 bp), *hsp65* (441 bp) and 16S rRNA (1506 bp) genes were identical for isolates LTA-388^T and 2003-11-06. The GenBank database was interrogated using a 189 nt sub-sequence of the *rpoB* gene sequence that corresponded to the 216 nt hypervariable region previously used in a multi-alignment analysis of 26 *Mycobacterium* species (Lee *et al.*, 2003). We found that our sequence was unique and that the most closely related sequences were from *M. fortuitum* and *M. mageritense* (identity score, 88%). A search of the GenBank database for similarity to the 441 bp *hsp65* sequence revealed that

Fig. 1. Mycolic acid profiles determined by HPLC for strains LTA-388^T and 2003-11-06 and four other RGM species. Strains: LTA-388^T (a); 2003-11-06 (b); *M. smegmatis* ATCC 19420^T (c); *M. mageritense* ATCC 700351^T (d); *M. wolinskyi* ATCC 700010^T (e); *M. goodii* ATCC 700504^T (f). ISTD, Internal high-molecular-mass standard.

Fig. 2. Dendrogram, based on 1217 unambiguous nucleotides of the 16S rRNA gene, showing the phylogenetic relationships of strains LTA-388^T and 2003-11-06 and 26 other *Mycobacterium* species. Similarity comparisons, multiple sequence alignments and phylogenetic analyses of 16S rRNA gene sequences with pre-compiled aligned 16S rRNA gene sequence data at RDP-II (Cole *et al.*, 2003). The dendrogram was rooted in the sequence for *M. tuberculosis* as an outlier. GenBank accession numbers are shown in parentheses. The scale bar represents 1 substitution per 100 nucleotide residues.

the sequence was unique and was most similar to sequences from *M. neoaurum* and *M. diernhoferi* (95 %).

A phylogenetic comparison of 16S rRNA gene sequences using 1217 aligned nucleotides is shown in Fig. 2. The sequence of the helix-18 region of the 16S rRNA gene of the study strains matched that of other RGM sequences. Four species shared a common group on the 16S rRNA gene dendrogram, the study strains being separate from the remaining 22 species evaluated (Fig. 2). We therefore compared *hsp65* PRA patterns for these four species (*M. hodleri*, *M. frederiksbergense*, *M. neoaurum* and *M. diernhoferi*) with strain LTA-388^T, but no similarities in restriction patterns using either *Bst*EII or *Hae*III were observed (Fig. 3).

The genotypic and phenotypic characteristics of the four isolates in this study distinguish these organisms from other *Mycobacterium* species. Although the two strains of this species were obtained from different continents, all four isolates were associated with either a cosmetic environment (nail salon) or an infection of a patient undergoing mesotherapy for a cosmetic purpose. Infections caused by RGM species acquired from aqueous baths at cosmetic salons are well documented (Gira *et al.*, 2004; Sniezek *et al.*, 2003; Murillo *et al.*, 2000; Winthrop *et al.*, 2002), and identification of these organisms is warranted.

The inability of the strains we studied to grow at 45 °C or upon media containing 5 % (w/v) NaCl are traits that may

be useful for distinguishing them from species belonging to the *M. smegmatis* complex, which are positive for these tests. Six additional physiological properties that can be used to distinguish strain LTA-388^T from those species found to share the greatest 16S rRNA similarities are shown in Table 2. Strain LTA-388^T differs from (i) *M. frederiksbergense*, which does not grow on MacConkey agar

Fig. 3. *hsp65* PRA patterns obtained using *Bst*EII and *Hae*III. Lanes: 1, strain LTA-388^T; 2, *M. hodleri* DSM 44183^T; 3, *M. frederiksbergense* NRRL B-24126; 4, *M. neoaurum* ATCC 25796; 5, *M. diernhoferi* ATCC 19340^T. S, 20 and 100 bp size standards.

Table 2. Physiological properties useful for differentiating strain LTA-388^T from closely related species

Strains/species: 1, LTA-388^T; 2, *M. frederiksborgense*; 3, *M. neoaurum*; 4, *M. hodleri*; 5, *M. diernhoferi*. Data for reference species were taken from Tsukamura *et al.* (1983) and Willumsen *et al.* (2001). Symbols: +, >85% of strains tested gave positive results; −, <15% of strains gave positive results.

Characteristic	1	2	3	4	5
Scotochromogenic	+	+	+	+	−
3-day arylsulfatase	−	−	+	−	−
Growth on MacConkey agar (no crystal violet)	+	−	+	−	+
Utilization of:					
Trehalose	+	+	+	−	−
Sorbitol	−	−	−	+	−
Citrate	+	−	−	−	−

containing no crystal violet or on citrate as a sole carbon source, from (ii) *M. neoaurum*, which is positive for 3-day arylsulfatase production and negative for citrate utilization, from (iii) *M. hodleri*, which does not grow on MacConkey agar containing no crystal violet, or on citrate or trehalose, but which does grow on sorbitol, as a sole carbon source, and from (iv) *M. diernhoferi*, which is non-scotochromogenic and does not grow on trehalose, sorbitol or citrate as a sole carbon source. Although the isolates had mycolic acid patterns that most closely resembled those of members of the *M. smegmatis* group, DNA sequence analysis of three taxonomically useful genes showed dissimilarities with respect to these or other recognized *Mycobacterium* species. The occurrence of unrelated strains that develop similar mycolic acid patterns has been described (Tortoli *et al.*, 2001). Moreover, the results for HPLC and genetic analysis are not always congruent, and the similarity, or lack of similarity, of HPLC patterns does not reflect phylogenetic relatedness of strains. The standard *hsp65* PRA method (Telenti *et al.*, 1993) showed restriction patterns (using *Bst*EII and *Hae*III) for the study strains that were easily distinguished from those of other RGM species.

We conclude, therefore, that strains LTA-388^T and 2003-11-06 represent two strains of a novel, and possibly pathogenic, member of the genus *Mycobacterium* that has characteristics that are clearly distinguishable from those of other species in this genus. We propose that this organism be named *Mycobacterium cosmeticum* sp. nov.

Description of *Mycobacterium cosmeticum* sp. nov.

Mycobacterium cosmeticum (cos.me'ti.cum. N.L. neut. adj. *cosmeticum* referring to cosmetics).

Cells are acid-fast rods (approx. 0.55 × 1.5 µm) that rarely form cell aggregates in liquid culture. Motility-related structures, spores and cell branching are not present.

Colonies on Middlebrook 7H10 or LJ agar have smooth surfaces and edges, are domed and are scotochromogenic. Colonies from a dilute inoculum on LJ agar are visible after 3 days incubation in an aerobic atmosphere at 28 or 35 °C, but no growth is observed at 45 °C. Growth occurs on MacConkey agar without crystal violet but not on LJ agar containing 5% (w/v) NaCl. Tests for niacin production and 3-day arylsulfatase activity are negative, but tests for 14-day arylsulfatase activity, nitrate reduction and iron uptake are positive. Tests for utilization of D-mannitol, D-xylose, iso-*myo*-inositol, L-arabinose, D-trehalose and citrate are positive, but that for D-sorbitol utilization is negative. Isolates are susceptible *in vitro* to ciprofloxacin, amikacin, tobramycin, cefoxitin, clarithromycin, doxycycline, sulfamethoxazole and imipenem. The mycolic acid HPLC pattern consists of two three-peak clusters and is most similar to patterns for *M. smegmatis*, *M. wolinskyi* and *M. goodii*. Partial sequences of the *rpoB* (321 bp), 16S rRNA (1506 bp) and *hsp65* (441 bp) genes are different from those of all currently recognized *Mycobacterium* species. Characteristics that distinguish this species from other RGM species include the inability to grow at 45 °C or on media containing 5% (w/v) NaCl, the utilization of trehalose or citrate, but not sorbitol, as a sole carbon source, and a unique banding pattern after restriction of a 441 bp region of the *hsp65* gene that includes major fragments of 310 and 130 bp (when *Bst*EII is used) or of 140, 96, 80 and 40 bp (when *Hae*III is used).

The type strain, isolate LTA-388^T (=ATCC BAA-878^T = CIP 108170^T), was isolated from a granulomatous lesion of a female patient in Venezuela who was undergoing mesotherapy.

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